Antimicrobial susceptibility pattern of Salmonella enterica, blood-stream isolates, among febrile children: a prospective study from Nepal

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Abstract

Background Still, in developing the children are being treated empirically and irrationally with accessible antibiotic without susceptibility testing and minimal lethal dose calculations, defying the probable MDR (multi-drug resistance) isolates. This study was undertaken in the febrile children to determine the antimicrobial susceptibility pattern of Salmonella enterica against commonly prescribed antibiotics.

Method All isolates were identified by biotyping and serotyping standard protocols then tested against antibiotics by modified Kirby disk-diffusion method. Minimum Inhibitory Concentration (MIC) of isolates were determined by agar dilution method and compared with disk diffusion results and on nalidixic-acid sensitive/resistant strains.

Result Among 1815 enteric-fever-suspects, 90(4.9%) isolates of Salmonella enterica [serovar: 62(68.8%) Salmonella Typhi and 28 (31.1%) Salmonella Paratyphi A] were recovered. The incidence of infection was higher among male, age group 5 to 9, and patient from the out-patient department (OPD). On disk-diffusion test most isolates, were sensitive against first-line drugs, cephalosporins, and macrolides. However, against quinolone, a huge percentile i.e. 93.3%, of isolates were resistant [including 58 Typhi and 26 Paratyphi serovar], and nearly 14% against fluoroquinolones. When MIC breakpoint was adjusted 4μg/ml for azithromycin, ≥1 μg/ml for ciprofloxacin, 2μg/ml for ofloxacin, 8μg/ml for nalidixic acid, 1μg/ml for cefixime, higher sensitivity and specificity achieved while screening decreased susceptibility. Among tested antibiotics, low rate of resistant strain observed on MIC of azithromycin. Also, higher resistance against fluoroquinolones observed on NARS strain.

Conclusion Higher susceptibility of Salmonella enterica to first-line drugs (the conventional antityphoidal drugs), third-generation cephalosporins, and azithromycin; advocates for its reconsideration in the implicated therapy. However, lower susceptibility against fluoroquinolones among nalidixic-acid resistant Salmonella (NARS) strain negates its empirical use in children.

Keywords Enteric fever, Nepal, children, Salmonella enterica

Background

Enteric febrile illness caused by *Salmonella enterica* species, the salmonellosis, accounts a burgeoning global threat—disproportionately affecting more than 17 million people with a recorded mortality 178 000 000 annually(1)(2). The exact data could be even dreadful since millions of unrecorded death due to febrile illness with unknown etiologies in developing countries come to limelight time and again. Apart from this, probable antimicrobial resistance (AMR) in the pathogen, is another obstinate challenge; however, if therapeutic approaches could be made accessible in these regions.

Salmonellosis is a predominant cause among blood-stream infection irrespective to any age categories; nevertheless, the higher incidence in children reflects its active community-acquired transmission(3)(4)(5). Not surprisingly, children in developing countries are still

being treated empirically and irrationally with traditionally ampicillin, ciprofloxacin, ofloxacin, chloramphenicol, and trimethoprim-sulfamethoxazole, without susceptibility testing and minimal lethal dose calculations, defying the probable AMR in the isolates(6). In these backdrops, to determine the antimicrobial susceptibility pattern of *Salmonella enterica* against commonly prescribed antibiotics in febrile children, the study was undertaken.

Materials And Methods

Study design and sample population

A cross-sectional study was conducted among enteric fever suspected children (up to 14 years of age) in International Children Friendship Hospital (ICFH), a tertiary care hospital for children, in Kathmandu, Nepal. The study was conducted over one year (April 2017-March 2018), where all *Salmonella enterica*, recovered from blood samples were included. The isolates, however, obtained from the sample (other than blood) and the same patient were considered as duplicated isolates, hence excluded. Also relevant patient information, brief clinical history, and history of antibiotic use (if under therapy), was taken. Data regarding personal information and infectious disease were coded and kept confidential.

Laboratory methods

The clinical suspicion of enteric fever was made by the respective unit pediatrician. The blood samples were collected aseptically (about 2-3ml) and cultured in brain heart infusion broth (HiMedia, India) as per guideline set by American Society for Microbiology(ASM) for conventional blood culture(7). Further, isolation and identification of the isolates were done by standard microbiological techniques—biotyping (colony morphology, staining reaction, and biochemical characteristics) and serotyping using specific antisera (Denka Seiken Co. Ltd., Tokyo, Japan)(7). The samples were considered sterile if no bacterial growth was observed on subculture after 7 days of aerobic incubation at 37°C.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of *Salmonella enterica* against antibiotics (commonly used in Nepal) was tested by the disk diffusion method [modified Kirby-Bauer method] on Mueller Hinton agar (Hi-Media, India) in compliance of standard procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), Wayne, PA, USA(8). The antimicrobials tested were: amoxicillin (10 μg), azithromycin(15 μg), cefixime(5 μg), ceftriaxone(30 μg), cephotaxime(30 μg), chloramphenicol(30 μg), ciprofloxacin(5 μg), cotrimoxazole(25 μg), nalidixic-acid (30 μg), ofloxacin(5 μg). The interpretations of susceptibility results were made based on interpretative zone diameters suggested by CLSI. For the standardization of susceptibility testing, *Escherichia coli* ATCC (American Type

Culture Collection) 25922 and *Staphylococcus aureus* ATCC 25923 were used as control organism.

Determination of Minimum Inhibitory Concentrations (MICs)

MICs of ciprofloxacin, ofloxacin, nalidixic-acid, azithromycin, and cefixime were determined by agar dilution method as suggested by Andrews(9) based on CLSI guidelines(8); and classified sensitive or resistant accordingly. Of the total 90 isolates, MICs value of only 71 isolates were determined by agar dilution method since 9 of the isolates were not preserved and 10 isolates could not be revived.

Comparison between disk diffusion test and MICs

The results of disk diffusion test and agar dilution test among five antibiotics (azithromycin, cefixime, ciprofloxacin, ofloxacin, and nalidixic-acid) were compared by WHONET 5.4 software.

Correlation of NARS and fluoroquinolones (FQs)

The obtained isolates were broadly classified to nalidixic-acid sensitive Salmonella (NARS) strains and nalidixic-acid sensitive Salmonella (NASS) strains and correlated against the resistance pattern of FQs.

Data management and analysis

The data obtained was entered in Microsoft Office Excel 2007 and analyzed by Statistical Package for Social Sciences (SPSS) version 16.0. The susceptibility data (with observed zone size) and MIC values of ciprofloxacin, ofloxacin, nalidixic-acid, azithromycin, and cefixime were analysed by WHONET 5.4 software.

Results

Patients' demographics

During the study period, a total of 1815 enteric fever suspected patients (including 997 male and 818 female patients) were enrolled. Of total suspects, the rate of infection was higher in male 55 (5.5%) compared to female 35 (4.2%). The highest number of the isolates were obtained from the patient's age group (in the range 5 to 9 years) and those enrolling in the out-patient department (OPD) and wards. Prior attending to hospital, 304 (218 on ciprofloxacin; 86 ofloxacin) had self-medicated history, they only visited in case where there was not symptomatic resolution (Table-1).

Bacterial isolates

Of total samples, 4.9% (n=90) *Salmonella enterica* isolates were recovered, where 62(68.8%) were *Salmonella* Typhi (S. Typhi) and the remaining 28 (31.1%) were *Salmonella* Paratyphi A (S. Paratyphi).

Antibiogram of *Salmonella enterica* isolates on disk diffusion test

On antimicrobial susceptibility testing with disk diffusion, most of the recovered isolates, were sensitive to first-line drugs (amoxycillin, chloramphenicol, and cotrimoxazole), third-generation cephalosporins (cephotaxime, ceftriaxone, and cefixime), and macrolides (azithromycin). However, on FQs (ciprofloxacin and ofloxacin), 12 isolates were resistant to ciprofloxacin and 13 to ofloxacin (Table-2). None of the isolates was MDR.

Moreover, higher resistance was attributed on quinolone—84 NARS strain were isolated. Of which, 58 were serovar S. Typhi while 26 were S. Paratyphi.

Antibiogram of *Salmonella enterica* based upon MICs

Susceptibility result of 71 isolates were tested for MIC of antibiotics: ciprofloxacin, ofloxacin, and nalidixic-acid, cefixime, and azithromycin are shown in Table-3. The sensitive/ resistance isolates were classified according to CLSI against MICs of antibiotics.

Furthermore, when MIC breakpoint was adjusted 4µg/ml for azithromycin, ≥1 µg/ml for ciprofloxacin, 2µg/ml for ofloxacin, 8µg/ml for nalidixic-acid, 1µg/ml for cefixime, the inhibition zone diameter of 19mm, ≤ 28 mm, 17 mm, 19 mm, 19 mm attained for respective antibiotics i.e. higher sensitivity and specificity in screening for decreased susceptibility yielded. Among tested antibiotics, low rate of azithromycin resistant strain was observed on MICs (Fig.1).

Indicators of NARS with FQs

The MICs of FQs (ciprofloxacin and ofloxacin) among NARS and NASS isolates are shown in the fig.2. Similarly, scatter plot correlating the MICs of ciprofloxacin, ofloxacin and nalidixic-acid against *Salmonella* isolates are shown in the (supplemental fig1 and fig.2). The simultaneous presence of reduced FQs susceptibility was observed in NARS.

Discussion

In most hospitals of Nepal, enteric fever or typhoid is one of the leading diagnosis of febrile illness; series of enteric fever outbreaks with changing antimicrobial resistance trend have been reported(10)(11)(12). The incidence in children (age categories up to 14 years) has been scarcely reported from Nepal; however, a pocket endemic region(13). In these perspectives, estimation of the disease burden and its etiologies along with antimicrobial susceptibilities are obligatory in an effective prevention and control interventions.

The overall incidence rate of enteric fever, in the pediatric population, caused by serovars of *Salmonella enterica* in our hospital was 4.9%. The incidence doubled in this interval of 11 years when Prajapati et al. 2008 reported 2.0% (14). Although the study population is different including all age categories; the incidence of salmonellosis was recorded up to 15.6% from rural areas of Nepal(15). Besides, the low rates of culture-positive enteric fever in our study probably due to self-medication prior hospital arrival (often practiced in rural areas of Nepal) and discrepancy in sample volume collected (particularly in infants and small kids where the required volume could not be drawn) as requires for culture. The higher number of isolates were recovered from the age group 5to 9 years (primary school-going children) in our study. The probable reason might be due to unhygienic behaviors—lacking knowledge of proper handwashing before a meal and after defecation.

Out of 90 culture-confirmed cases of salmonellosis, 62(68.8%) were caused by S. Typhi and the remaining 28 (31.1%) were S. Paratyphi A. The predominance of serovar Typhi act in accordance with the observations made by Zellweger et al. 68.5% and 30.5% and Petersial et al. 55.7% and 44.3% respectively for serovars S. Typhi and S. Paratyphi (16)(13) Shirakawa et al., nevertheless, reported S. Paratyphi as more prevalent serovar(17); his finding is corresponding to Pramod et al. (35.9% S. Typhi and 64.1% S. Paratyphi)(18). There is no such well-established reason behind this variation of serovars; however, it can be assumed, the higher incidence of Typhi could be achieved via water-borne transmission as requires smaller inocula than paratyphoid which requires larger inocula via food-borne transmission(12)

FQs and nalidixic-acid, owing to easily accessible (even sold from the medical pharmacies without prescription), less expensive, and availability in oral forms, are established as the mainstay of therapy against salmonellosis—particularly in developing countries like Nepal(13)(19) (16). Although, with the emergence of NARS strains around the globe, their efficacy against enteric fever is now questionable(20)(21). Among enrolled children, 304(218 on ciprofloxacin; 86 on ofloxacin) had self-medicated history (treated with-out knowing etiologies and drug resistance pattern). However, we observed a very high level of FQs and nalidixic-acid resistance, but relatively low rates to conventional drugs. The findings are in line with recent epidemiological studies conducted in the Nepalese population(11)(13)(14)(16).The low rate of resistance to conventional drugs was observed, probably due to discontinuation in the therapeutic regimen for a longer time, high molecular weight self-transmissible plasmid inducing resistance could have lost or de novo susceptible strain might have emerged in these days(11)(22). Due to mutation in the genes coding for DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE), the high level of nalidixic-acid resistance occurs, in general (23). However, lower susceptibility to fluoroquinolones possibly occurs owing to the enhanced active efflux and early overproduction of the AcrA pump in isolates with the gyrA mutation(23).

In our study, among tested antibiotics, low rate of azithromycin resistant strain was observed on MIC which is similar as Khanal et al. reported such efficacy—lower MIC and resistance trend against the isolates, in his study(22). In the Nepalese population, treatment failure on azithromycin treatment is yet not reported; nevertheless, an increase in MIC was reported in the patients from other countries. Relying upon this background, we advocate for its choice as implicated therapy against salmonellosis.

Besides, the third-generation cephalosporins (ceftriaxone, cefotaxime, and cefixime) had shown an excellent effectiveness against Salmonella serovars with sensitivity up to 100% (24)(25). In our study, 98.8% isolates were sensitive against cephalosporins supporting the efficacies.

Moreover, MDR Salmonella isolates with fluctuating resistance trend have been increasingly reported from Asian countries(10)(17)(26)(27). In our study subjects, fortuitously, no MDR salmonella isolates was recovered though have been reported earlier from Nepal(16).

Limitations

We could not evaluate the risk factor and treatment outcomes in our settings (only in-vitro susceptibility testing were done); although, a hospital-based study. Further clinical evaluations could be more elucidative if a large number of samples were included in our study. Moreover, lacking the molecular laboratory set-up (presumed as a necessity for high-quality data in clinical studies) was the major drawback of our study since blood culture have limited sensitivity.

Conclusions

Regardless of surging drug-resistant *Salmonella enterica* cases elsewhere, the level of resistance was not as high in our study population as predicted. MDR trend may vary, therefore drugs susceptibility testing side-by-side to empirical therapy is mandatory. Referring to our findings, higher susceptibility of *Salmonella enterica* to first-line drugs (the conventional antityphoidal drugs), third-generation cephalosporins, and azithromycin; and we advocate for its reconsideration as an implicated therapy against salmonellosis. Nevertheless, the decreased susceptibility against fluoroquinolones among nalidixic-acid resistance strain negates its empirical use in children.

Declarations

Abbreviations

AMR: antimicrobial resistance; ASM: American Society for Microbiology; ATCC: American Type Culture Collection; CLSI: Clinical and Laboratory Standard Institute; MDR: multiple-drug resistant; NARS: nalidixic-acid resistance Salmonella; NASS: nalidixic-acid sensitive Salmonella; S. Typhi: Salmonella Typhi; S. Paratyphi: Salmonella Paratyphi

Authors' contributions

PK and JT made the diagnosis, designed the manuscript, reviewed the literature and prepared the article for submission. ST helped for literature review, gave concept of research paper and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no competing interests.

Availability of data and materials

Data generated or analyzed during this study are included in this manuscript and remaining are available from the corresponding author on reasonable request.

Consent to publish

Not applicable.

Ethics approval and consent to participate

This research was approved by the Institutional Review Committee of International Friendship Children's Hospital, Kathmandu, Nepal. A written informed consent was taken from their parents before participating in the study. Data regarding personal information and infectious disease were coded and kept confidential.

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Tables

Due to technical limitations, the tables are available in the supplementary section.

Figures

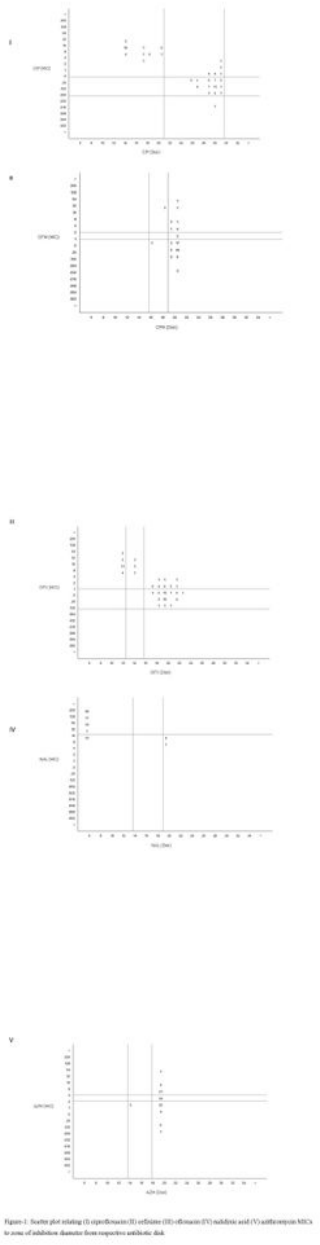

Figure 1

Scatter plot relating (I) ciprofloxacin (II) cefixime (III) ofloxacin (IV) nalidixic acid (V) azithromycin MICs to zone of inhibition diameter from respective antibiotic disk

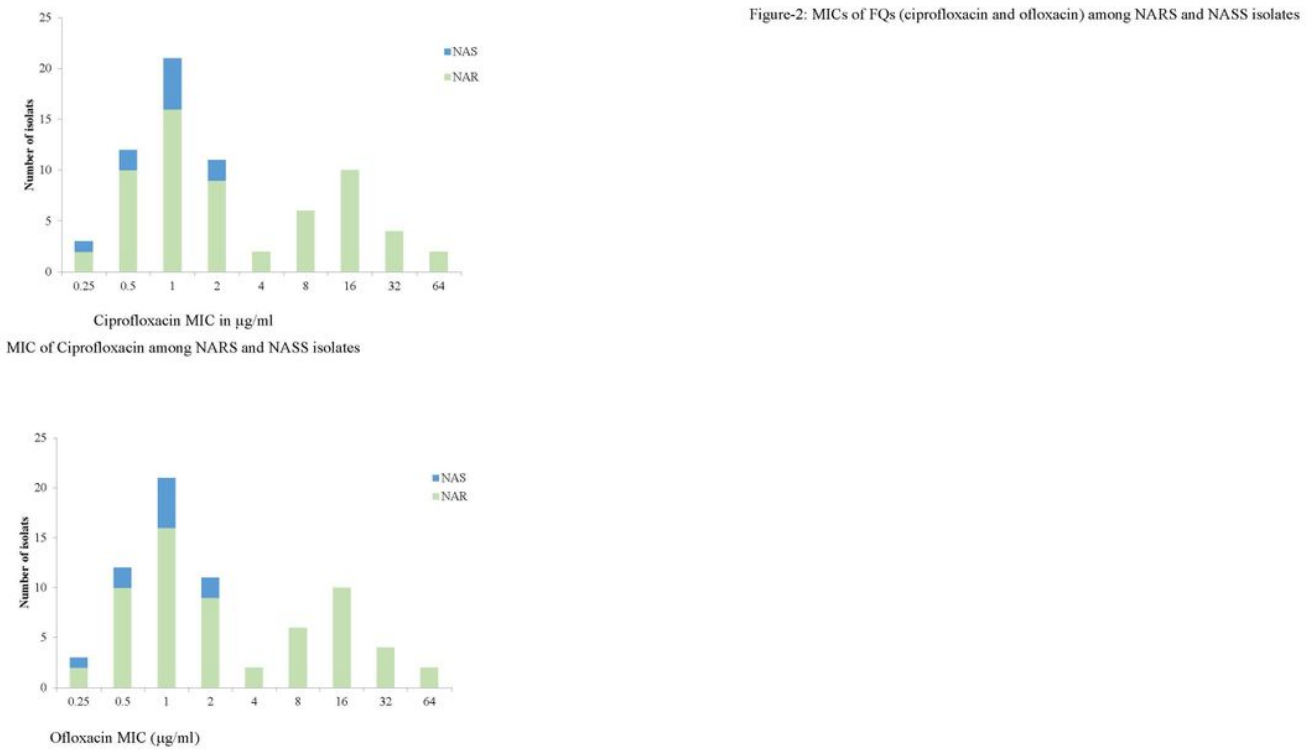

Figure 2

MICs of FQs (ciprofloxacin and ofloxacin) among NARS and NASS isolate

Supplementary Files

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- SupplementalFigure.docx